# STUDIES ON BEHAVIOR OF CHROMOSOMES AND DEVELOPMENT OF EMBRYOSACS AND POLLEN GRAINS IN HAPLOID RICE PLANT

By
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## I. Introduction

Haploid rice plant was discovered first by Morinaga and Fukushima (1931), and was reported successively by Nakamura (1933), Ramiah et al (1933) and others. Occurrence of haploid plants has been regarded by those workers as an important discovery, because it is usefull for studying various genetical problems related to the genom constitution of the rice plant.

Nakamura (1933) reported that in haploid rice, 12 univalent chromosomes did not pair each other and were distributed on the spindle at random at the first metaphase and anaphase in meiosis, while the other workers (Morinaga 1934, Jones and Longley 1941) found that one or two bivalents were formed in haploid rice.

In order to clarify this point, pairing of chromosomes and gametogenesis were studied. This work was carried out in the Taiwan Provincial College of Agriculture and in the Taiwan Agricultural Research Institute from 1950 to 1953. The writers express their hearty thanks to Dr. H. Oka and Mr. T. D. Chang who gave us the valuable materials, kind guidance and rivision during that time.

## II. Materials and Methods

A haploid plant was found in the Taiwan Provincial College of Agriculture by Dr. H. Oka from an Indian variety (P. T. B. 10) when the latrer was used as the maternal parent of a cross and the pollination was not successful. Another haploid plant preserved in the Taiwan Agricultural Research Institute was presented by the National Agricultural Research Bureau in Nanking. These haploid plants had narrow leaves and small spikelets and were sterile. A comparison of characteristics between the latter haploid plant and the diploid plant derived from seeds occasionally set by the hapoid plant is given in Table 1.

Table 1. Comparison of morphological characters between haploid and diploid plants.

| Strains | Plant height | Leaf width | Ear length | Num. of grains per ear | Num. of grains per cm. | Grain length | Grain width |
|---|---|---|---|---|---|---|---|
| | cm. | cm. | cm. | | | cm. | cm. |
| Haploid | 47.50 | 0.58 | 11.95 | 63.40 | 5.31 | 0.50 | 0.20 |
| Diploid | 86.00 | 1.23 | 20.15 | 89.20 | 4.43 | 0.80 | 0.30 |
| Ratio(x/2x) | 0.55 | 0.47 | 0.59 | 0.71 | 1.20 | 0.63 | 0.66 |

For observing pollen mother cells and gametogenesis, the anthers were fixed with Bouin's fluid, and paraffin section method was used. (Heidenhain's Iron-Alum Haematoxylin Stain).

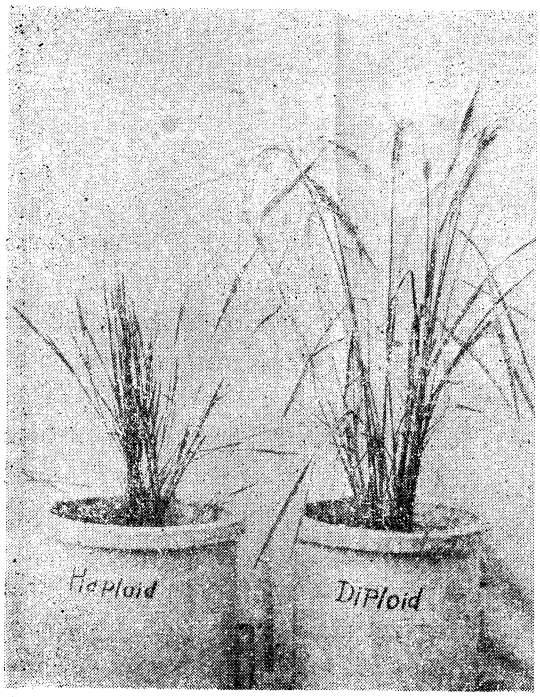



Fig. 1. Haploid and diploid rice plant standing in pots.

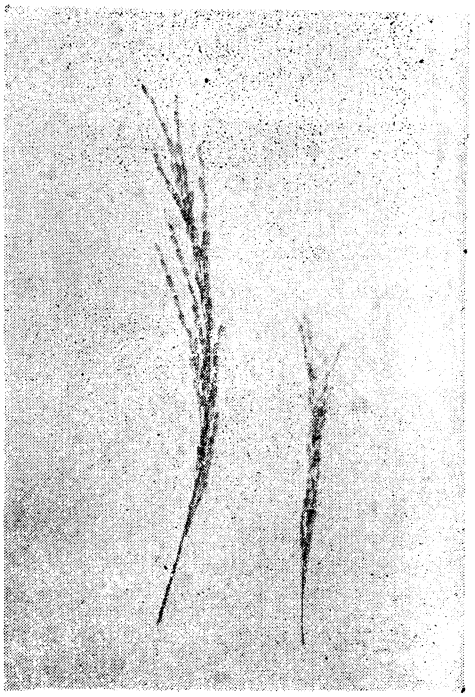


Fig. 2. Panicles of diploid and haploid rice plants.

## Ⅲ. Observation Results

1. Meiosis in Pollen Mother Cells.

12 thread-shaped chromosomes were found at the diplotenestage. At diakinesis and first anaphase, one bivalent or two univalents were found to associate with each other end to end as seen in Figs. 3 and 4. At the first anaphase, 12 univalents were found to be scattered on the nuclear plate. The frequency of nuclei with one or two bivalents at diakinesis and first metaphase is given in table 2.

Table 2. Frequency of different types of chromosome pairing in haploid rice.

| Stages / Types of pairing | Diakinesis | 1st Metaphase |
|---|---|---|
| 2Ⅱ+8Ⅰ | 2 | 17 |
| 1Ⅱ+10Ⅰ | 5 | 16 |
| 12Ⅰ | 5 | 17 |
| 1Ⅱ+9Ⅰ+2(0.5) | — | 3 |
| 1Ⅱ+8Ⅰ+4(0.5) | — | 1 |

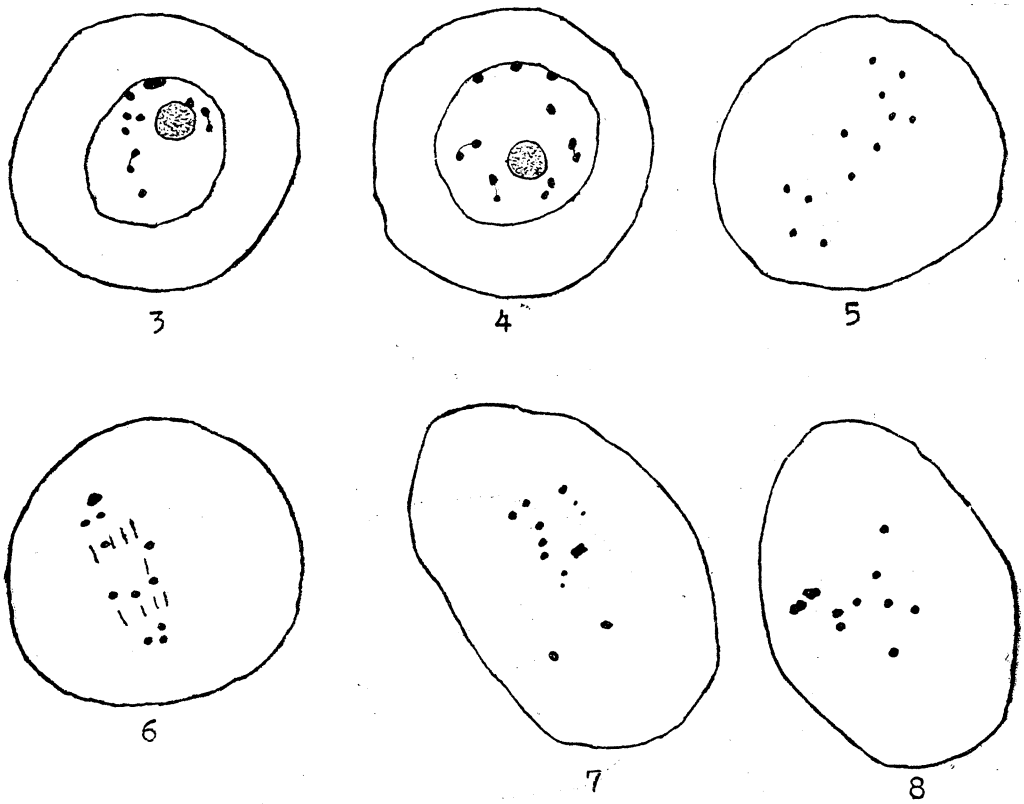
| | | |
|---|---|---|
| 11 I +2(0.5) | — | 3 |
| Total | 12 | 57 |

As seen in Table 2, "12I" and "1II+10I" were higher in frequency than the other pairing types. Further, at the first metaphase, some univalents came near each other as if it were a secondary pairing.

Table 3 shows the frequency of nuclei with such seemingly paired univalnts. From such a phenomenon, those univalents seem to have homology among them.

Table 3. Frequency of association between univalent chromosomes at 1st metaphase.

| | Groups of different num. of associated univalents | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1×2 | 1×3 | 2×2 | 2×3 | 3×2 | 4×2 | |
| Num. of cases observed | 24 | 10 | 2 | 10 | 3 | 6 | 2 | 57 |




Figs. 3-8. Nuclei at Diakinesis and 1st Metaphase in PMCs. of haploid rice plant.
3. Diakinesis showing 1 bivalent and 10 univalents.
4. Diakinesis with 12 univalents.
5. 1st Metaphase with 12 unvalents.
6. 1st Metaphase showing 1 bivalent and 10 univalents.

7. 1st Metaphase showing 1 bivalent. 8 univalents and 4 splitted chromosomes.
8. 1st Metaphase showing 2 bivalents and 8 univalents.

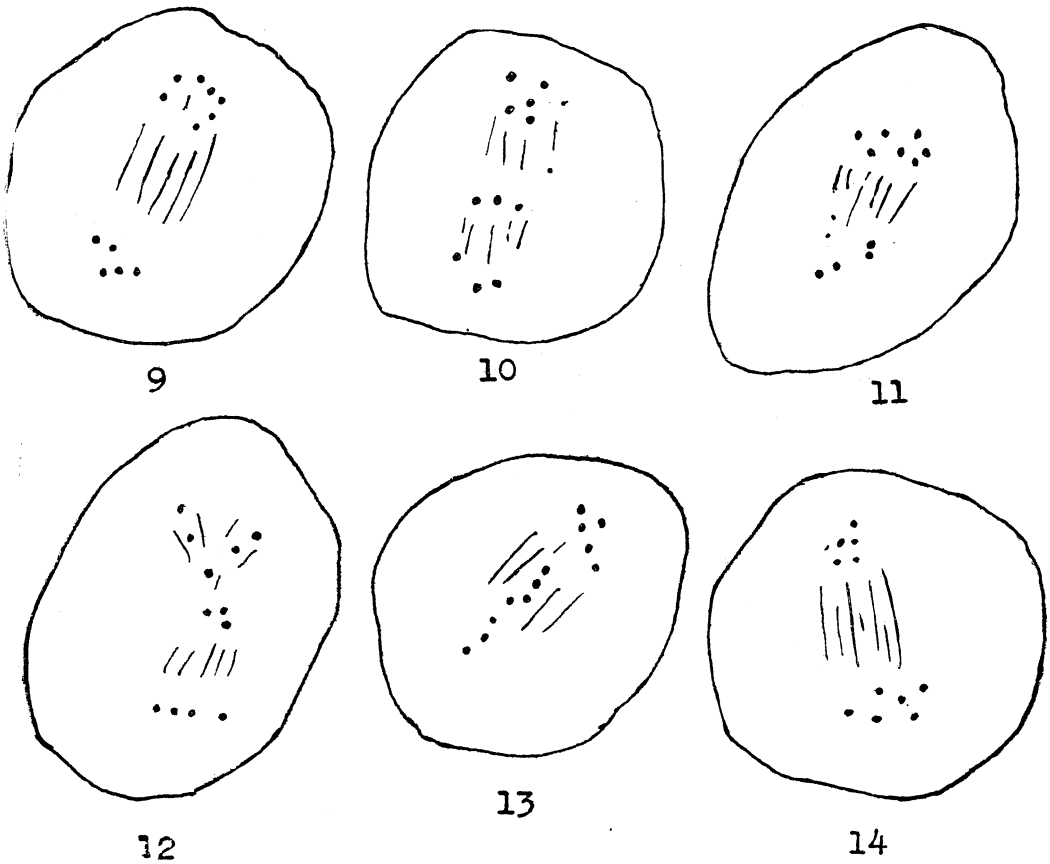
ca. ×2000

At the first anaphase, the univalents seemed either to be distributed at random to the poles (Figs. 9-14) or to split themselves, though the latter case was rare. The frequency of different types of segregation at the first anaphase is given in Table 4. As seen in Table 4, the 12 chromosomes were divided into 6 and 6 in a majority of cases.

Table 4. Types of division of univalent chromosomes at the first anaphase.

| Types of chromosome segregation | 6 : 6 | 7 : 5 | 8 : 4 | 9 : 3 | 10 : 2 | Num. of cells observed |
|---|---|---|---|---|---|---|
| Frequency | 15 | 7 | 5 | 1 | 1 | 29 |

In many nuclei, the second division was not found. This may be possibly due to the irregularity of the first division. At the tetrad stage, then, normal tetrads were formed in only a few cases, and in a greater part of cases, groups of two or three cells were found.




Figs. 9-14. 1st anaphase in pollen mother cells of haploid rice plant.

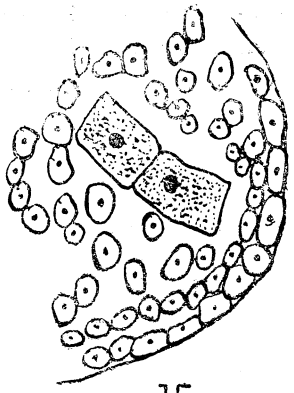
| | | | |
|---|---|---|---|
| 9. 7 : 5 | 10. 6 : 5+2(0.5) | 11. 7 : 4+2(0.5) | |
| 12. 8 : 4 | 13. 5 : 7 | 14. 6 : 6 | |

ca. ×2000

2. Embryosac Formation

It was found that the division of embryosac mother cells began a little later than that of pollen mother cells. In a greater part of ovaries observed, two or three megaspores (Figs. 15-16) were found, but the normal tetrad was not found after meiosis. Considering this fact, the second division seems to proceed normally in only a few ovaries. All of those megaspores were found to deteriorate soon, while the ovary seemed to grow normally.

At the time of anthesis, a trace of degenerated embryosac, which stained deeply, was found, but no embryosac cavity was seen in the ovary; the ovary was filled uniformly with nucellus-like cells (Fig. 18). In contrast, it is known that the embryosac cavity of a sterile plant even have no normal part of an embryosac.



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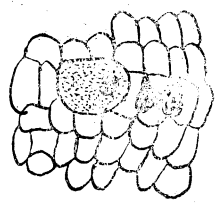

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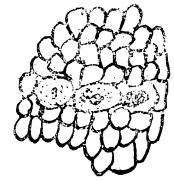

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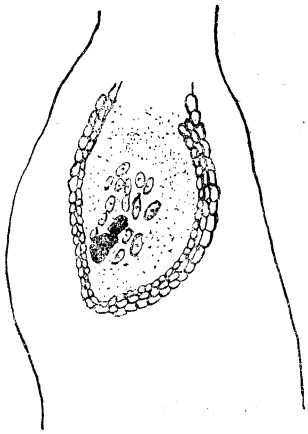

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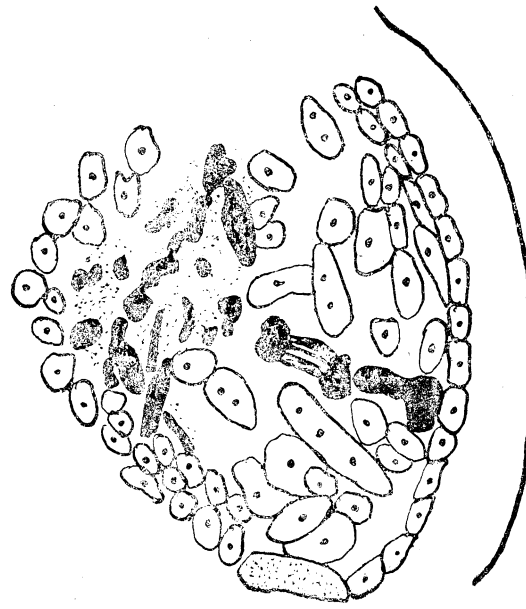

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Figs. 15-19. Embryosacs of haploid rice.

15. Dyad of embryosac, possibly unable to form a normal tetrad. ca. × 800
16. Two degenerated megaspores out of three. ca. × 800
17. Triad began to degenerate at the same time. ca. × 800
18. Degenerated dyad after the division; the trace is very clear. ca. × 600
19. Degenerated triad after the division, the trace of which can be seen in nucellus cells. ca. × 2000

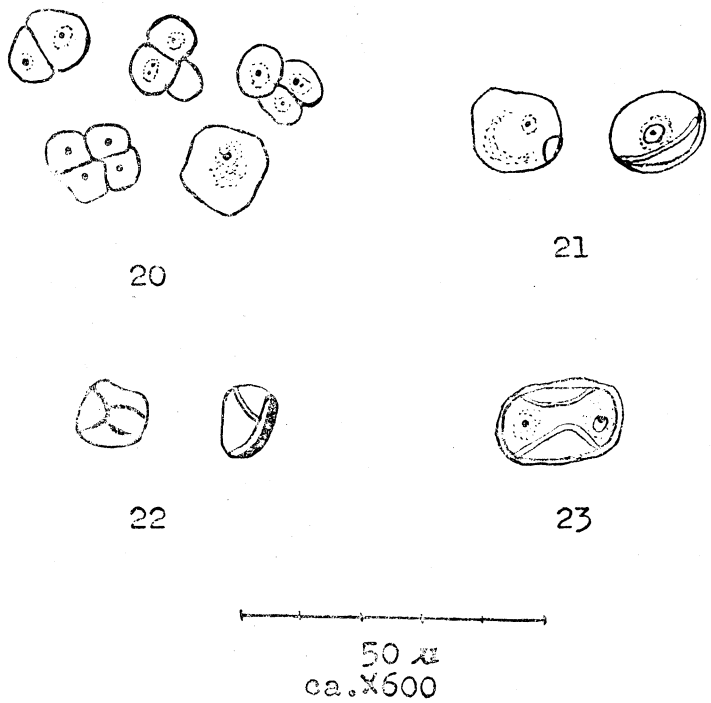
3. The Development of Pollen Grains.

At the end of meiosis, group of three or two microspores were found, besides apparently normal tetrads (Fig. 20) with frequencies given in Table 5.

Table 5. Frequency of the groups of microspores in different number at the tetrad stage in haploid rice.

| Num. of microspore within a group | 1 | 2 | 3 | 4 | ? | Total |
|---|---|---|---|---|---|---|
| Num. of cases observed | 7 | 20 | 9 | 10 | 7 | 53 |

The diameters of these microspores vary from 5$\mu$ to 10$\mu$. These microspores usually showed one nucleus, containing one or two nucleoli, but sometimes none. In one microspore, further, numerous small nucleoli, in addition to two large and three medium ones,




Figs. 20-30. The development of pollen grains of haploid rice.
20. Tetrad, triad, dyad and monad of haploid rice.
21. Normal pollen grains formed after 1st contracting stage.
22. Degenerated pollens.
23. The giant pollen grain with two nuclei.

were observed. Such microspores may not have received a complete set of chromosomes.

After this stage, microspores were separated from each other in the usual manner and increased their diameters slightly. When the development stepped into the so-called first contracting stage, the diameter of microspores was 11$\mu$ to 18$\mu$.

Passing through this, some microspores were gradually transformed into a spherical shape, showing seemingly normal nuclei (Fig. 21) but others seemed to degenerate (Fig. 22). The large microspores, about 30$\mu$ in diameter, were found in a few cases. A giant microspore possessing two nuclei was also found.

The microphyle was formed in some microspores, but it was not clear whether all microspores formed the micropyle or not. The pollen grains seemed to possess only one layer of membrane,

At the second contracting stage, all microspores seemed to degenerate. Anthers did not break at the heading stage, and all of the pollen grains were empty.

## Ⅳ. Discussion

Up to date, haploid plants have been reported in many plant species. According to Jørgensen (1928), haploid plant in *Solanum* is due tothe parthenogenetic development of egg nucleus; which may have been stimulated by germ nuclei from pollen. In this case, the germ nucleus may enter the embryosac, but may fail to fertilize the egg nucleus. In cereals, it is known that haploid plants usually arise from the failure of interspecific crosses.

Generally, the association of chromosomes is considered to be due to homology between them. In a haploid plant, since the chromosomes have, as a rule, no homologus parts between them, no association can take place in meiosis. This was affirmed in many haploid plants derived from diploid species (*Datura stramonium*, Belling and Blakeslee 1923, 1927; *Crepis capillaris*, Hollingshead 1930).

But, in haploids which arise from allopolyploid plants, bivalents can occasionally be observed possibly due to affinity between the corresponding chromosomes belonging to different genoms (*Brassica napella*, Morinaga and Fukushima 1933; *Nicotiana tabacum*, Clausen and Mann 1924).

In haploid rice, Morinaga and Fukushima (1927) and Jones and Longley (1941) found one or two bivalent chromosomes at the first metaphase. This phenomenon was recognized also in the present study (Table 6).

Table 6. The number of bivalent chromosomes at diakinesis and first metaphase.

| Total num. of chromosome<br>Num. of bivalent | 11 10 9 8<br>1 2 3 4 | Num. of cells observed | Mean num. of bivalent |
|---|---|---|---|
| Diakinesis | 5 2 — — | 12 | 0.75 |
| 1st Metaphase | 20 17 — — | 57 | 0.95 |
| Total | 25 19 — — | 69 | 0.91 |

In the present study, terminal association of chromosomes was found in diakinetic nuclei. This may account for the existence of bivalent chromosomes at metaphase. If we consider rice as a secondary polyploid plant as suggeseted by Sakai (1935) as well as by Nandi (1936), such an association will correspond to the "secondary association" in diploid. In contrast, if we consider rice as possessing many structural changes of chromosomes, the formation of bivalent in haploid plants may indicate the existence of segmental duplications.

The haploid rice was completely sterile due to the failure of meiosis as described in the present study. However, rarely, a few seeds were set possibly due to mutual fertilization of unreduced gametes. Such a diploid plant may be valuable for various genetical studies, since it is completely pure in genetical constitution.

## V. Résumé


1. The present paper describes the behavior of chromosomes in meiosis and the development of embryosacs and pollen grains in haploid rice plant.
2. Two haploid plants were observed; in both of them, pollen mother cells at diakinesis of first metaphase were found to have one or two bivalent chromosomes. This seems to suggest that certain chromosomes in haploid set are to some extent homologus with each other.
3. The second division was not found in a majority of pollen mother cells. This seems to be due to the failure of the first division.
4. In a larger part of cases, two or three megaspores were formed from an embryosac mother cell and they seemed to degenerate soon. No normal embryosac tetrad was found.
5. The number of microspores produced from one pollen mother cell varied from two to four.
6. The haploid plants were completly sterile due to the failure of meiosis such as mentioned above.


## VI. References


1. HOLLINGSHEAD, L., 1930. A cytological study of haploid Crepis capillaris plants. Univ. Calif. Pub. Agr. Sci. 6: 107-129
2. JONES J. W. and Longley. A. E., 1941. Sterility and aberrant chromosome number in Caloro and other varieties of rice. Jour. of Agr. Res. 62(7):381-399
3. JØRGENSEN C. A., 1928. The experimental formation of heteroploid plants in the Genus Solanum. Jour. of Gen. 19: 133-211
4. KIHARA H., 1942. The pollen development of rice plant (in Japanese) Agr. and Hort. 17(6): 685-689
5. KUWADA, Y., 1910. A cytological study of Oryza sativa L. Bot. Mag. 24 Tokyo
6. LAWRENCE, W. J. C., 1931. The secondary association of chromosomes. Cytologia 2
7. MORINAGA, T. and FUKUSHIMA E., 1931. Preliminary report on the haploid plant of rice, Oryza sativa L. Proc. of Imp. Aca. 7(10): 383-384

8. _____1934. Studies on the haploid plant of Oryza sativa L. Jap. Jour. of Botany. 7: 35-105
9. _____1933. Karyological studies on a spontaneous haploid plant of Brassica napella. Cytologia 4: 457-560
10. _____1939. Cytogenetics on Oryza (in Japanese). Botany and Zoology 7: 197-201
11. NAKAMURA, S., 1933. The haploid plant in rice (in Japanese). Jap. Jour. Gen. 8: 223-227
12. NANDI H. K., 1936. The chromosome morphology, secondary association and origin of cultivated rice. Jour. of Gen. 33: 315-336
13. RAMIAH, K., PARTHASARATHY, N. and RAMANUJAM, S., 1933. Haploid plant in rice (Oryza sativa). Current Sci, Mysore 1: 277-278 (Plant Breeding Abst. 3(4): 1933).
14. SELIM. A. G., 1930. A cytological study of Oryza sativa L. Cytologia 2: 1-26
15. SAKAI, K. I., 1935. Chromosome studies in Oryza sativa L. I The secondary association of the meiotic chromosome (in Japanese) Jap. Jour. Gen. 11 145-155
16. TERADA, S. 1928. Embryological studies in Oryza sativa L. Jour. of the college of Agr. Hokkaido Imp. Univ. 14: 41
17. YANG, L. C., 1947. Studies on embryosac development and fertilization in rice (in Chinese). Jour. of Agr. Asso. China. 185

# 單元體稻的染色體行動與胚囊及花粉粒發生之研究

謝順景　張文財


## 摘　要

1. 本研究係觀察單元體稻染色體之行爲，胚囊及花粉粒發生之過程，藉以解釋其不稔性之原因以及考察稻染色體組（genom）之構造者。
2. 在花粉母細胞之肥厚期與第一中期，可認出1～2個之双價染色體（bivalent）。由單價染色體間之接合現象，可推想，在單元體之12個單價染色體之間亦有若干相同染色體之存在。
3. 在大部份之情形下，胚囊母細胞在分裂直後形成兩個或三個大胞子，而此等大胞子不久卽開始退化。正常胚囊則未能發現之。
4. 在花粉發生之過程中，觀察相當於四分子之時期時，除可認出正常四分子之外，尙可認出三分子、二分子及一分子。
5. 單元體稻完全不結實，其主要原因可歸於減數分裂之失敗。